throughout the specification and, for example, on page 8, lines 14-16. Claim 10 has been amended to remove reference to internal organs. Claim 12 has been added. It is believed that no new matter has been introduced by these claim amendments. Various claims stand rejected under 35 U.S.C. § 112, paragraphs 1 or 2, 35 U.S.C. § 102(e) and 35 U.S.C. § 103(a). Each rejection will be addressed below. In light of the discussion below, it is believed that the above rejections of record have been overcome and that all pending claims are in condition for allowance. Action towards this end is respectfully requested.

I. Finality of the Action

Applicants note the Office Action is a Final Office Action and request the Examiner to remove the finality of the Action for the following reason. The Examiner has introduced a new ground of rejection under 35 U.S.C. § 102(e) that was neither necessitated by applicant's amendment of the claims nor based on information submitted in an information disclosure statement filed during the period set forth in 37 C.F.R. § 1.97 with the fee set forth in 37 C.F.R. § 1.17(p). In an Interview Summary mailed July 22, 2002 regarding a telephonic interview between Examiner Rawlings and applicants' attorney, Examiner Rawlings indicated that, with respect to the 35 U.S.C. § 102 rejection in the preceding Office Action (mailed November 26, 2001), "the prior art was improperly applied as the prior art was available under 102(e), not 102(b)." Examiner Rawlings stated that "the grounds of rejection are necessarily to be restated under 102(e) in a subsequent *non-final* action." (Emphasis added). Removal of the finality of the Office Action is respectfully requested.

II. Rejections under 35 U.S.C. § 112, second paragraph

Claims 1, 5, and 7-11 stand rejected as it is asserted that the phrase "comparing the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with the degree of the metachromatic shift of the dye from a library" is vague and indefinite. It is specifically asserted that a) it is not clear how the

spectra are to be compared ("not clear what aspect or characteristic of the spectrum are to be compared with the library of previously obtained spectra"); and b) the terms "metachromatic shift" and "the degree of the metachromatic shift" appear not to be defined in the specification. Applicants assert that the pending claims would be sufficiently definite to the skilled artisan with respect to the above-referenced recitation.

One skilled in the art is familiar with the phenomenon of metachromasia where, for example, a dye stains certain cell components a different color than the original color of the dye (also explained on page 3 of the application, lines 20-25). One skilled in the art would know how to quantitate the metachromasia by measuring the extent of the metachromatic shift by comparing the intensity of light in a desired light spectrum between, for example, two or more specific wavelengths. Notwithstanding the knowledge of the skilled artisan, the specification clearly teaches on, for example, page 21, line 29 to page 22, line 5, that the metachromatic shift is compared between two or more wavelengths. Therefore, the terms in the rejected claims are sufficiently definite to the skilled artisan.

Claims 1, 5 and 7-11 further stand rejected as it is asserted the phrase "with a library of previously obtained spectra of similarly stained tissue or cells" renders the claims vague and indefinite as it is asserted that it can not be determined from which similarly stained tissue or cells the library of previously obtained spectra is to be obtained prior to steps (a)-(d) and from what source the similarly stained tissue and cells are to be derived. The recited phrase would be clear to the skilled artisan.

For example, upon reviewing the specification, one skilled in the art would be aware that the library of previously obtained spectra would include those cells and/or tissues that are the subject of the particular analysis and are obtained from individuals that may exhibit the particular cellular abnormality. For example, if a diagnosis is to be made of skin cancer, analyses/spectra of such skin cancer cells/tissues obtained from individuals with skin cancer could serve as the particular reference. Therefore, the

recited phrase above relating to the library of previously obtained spectra of similarly stained tissue or cells would be clear to the skilled artisan.

Claims 1, 5, and 7-11 also stand rejected as it is asserted the phrase "correlating the reflected light spectrum with a disease state" is vague and indefinite as it is asserted that it can not be ascertained how the correlating the reflected light spectrum with a disease state leads to a diagnosis of dysplasia, pre-cancer or cancer in a living organism. It is more specifically asserted that it is unclear how the method is to be used to meet the objective recited in the preamble of the claim. It is further queried "how is the disease state to which [the] reflected light spectrum [is] to be correlated related to dysplasia, pre-cancer, or cancer?" Applicants respond that the pending claims are sufficiently definite to the skilled artisan with respect to the recitation "correlating the reflected light spectrum with a disease state."

For example, as explained in the specification, the stain taken up by diseased cells, such as those found in dysplasia, pre-cancer and cancer, stained according to the methods described therein, will exhibit a metachromatic shift, the extent of which can be determined from spectrophotometrically-obtained data. As clearly explained in the specification at, for example, page 22, lines 1-5, and in claim 1, one can compare the extent of the metachromatic shift in the test sample spectra with that obtained from the library of previously obtained spectra of similarly stained tissue or cells. The samples upon which the library is based have been previously diagnosed by conventional techniques as described in the specification and thus a correlation as to disease state of the test sample may be made by such comparison of the metachromatic shift of the test sample with that of the reference sample. Notwithstanding this, applicants have amended claim 1 to clarify the disease state is selected from dysplasia, precancer or cancer in a sincere attempt to advance prosecution.

Claims 1, 5, and 7-11 are asserted to be indefinite as claim 1 recites the term "similarly stained tissue or cells" and it is asserted that it can not be ascertained how similarly the different tissue or cells must be stained. Additionally, it is asserted claim

10, which recites the limitation "wherein the tissues or cells are from at least one organ selected from the group consisting of skin, cervix, vagina, mouth, colon, esophagus and internal organs," is indefinite as at least the cervix, colon and esophagus are internal organs.

With reference to the term "similarly stained tissue or cells", as histology is a mature art, the skilled artisan would be well aware that, in order to compare staining results from separately stained tissue/cell samples, the tissue or cells should be stained according to the same protocol. This would include, for example, use of the same dye at substantially the same concentration and staining for substantially the same amount of time.

With reference to the rejection of claim 10, applicants have amended claim 10 to remove reference to internal organs and have added new claim 12 which refers to internal organs. Withdrawal of the rejections of claims 1, 5, and 7-11 under 35 U.S.C. § 112, second paragraph, is respectfully requested.

III. Rejections under 35 U.S.C. § 112, first paragraph

Claims 1, 5, and 7-11 stand rejected under 35 U.S.C. § 112, first paragraph, as it is asserted that claim 1 recites "the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells" and "the degree of the metachromatic shift of the dye from a library," and that there does not appear to be proper and sufficient antecedent basis in the specification for recitation of these phrases, terms and/or limitations in the claims. It is further asserted that the recitations appear to introduce new matter, but the matter might be resolved if applicants point to particular passages in the specification that provide the necessary support for the recitations.

As mentioned on page 6, lines 15-17, of the March 5, 2002 response to the Office Action mailed November 26, 2001, support for the terms "the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or

cells" and "the degree of the metachromatic shift of the dye from a library" may be found, for example, on page 8, lines 9-16, and page 22, lines 1-5 and is not believed to add new matter. Withdrawal of the rejection of claims 1, 5, and 7-11 under 35 U.S.C. § 112, first paragraph, is respectfully requested.

IV. Rejection under 35 U.S.C. § 102(e)

A. Status of the case

Claims 1, 5 and 7-11 stand rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 5,784,162 to Cabib et al., as evidenced by Vaezy, et al. (*Journal of Microscopy* 163:85-94, 1991) and Marchesini, et al. (*Photochemistry and Photobiology* 55:515-522, 1992) for the reasons set forth in the previous Office Action mailed November 26, 2001.

In the previous Action, the following was asserted. Cabib et al. was relied on in the Action mailed November 26, 2001 for teaching spectral imaging methods for *in situ* medical diagnosis and treatment comprising preparing a sample to be imaged, viewing the sample through an optical device optically connected to a spectrometer, collecting and measuring incident light using a detector and collecting and interpreting data using a mathematical algorithm. It was further asserted in the Action mailed November 26, 2001 that 1) "numerous examples of *in situ* analyses of cells and/or tissues to either classify and/or diagnose cellular abnormalities in said cells and/or tissues are provided" in Cabib et al., and Examples 1, 6, 7 and 8 are pointed to with particularity; 2) Cabib et al. "discloses that a metachromatic dye, such as Azure-B, which is a thiazine dye, can be used to practice the prior art methods (see Example 2, column 43, line 10);" 3) "the sample of tissue or cells to be analyzed [in Cabib et al.] is prepared by staining with either Romanowsky-Giemsa stain, haematoxylin-eosin stain, or May-Grunwald-Giemsa stain (see claim 59), each of which are compositions comprising thiazine dyes;" 4) Cabib et al. teaches "that a spectral component may 'correlate well with what is called the purple Romanowsky-Giemsa complex,'" and 5) "an objective of the prior art

invention is to distinguish cancer from healthy or otherwise diseased tissue or cells (column 6, lines 27-33)."

Applicants respectfully traverse the various rejections for the reasons set forth in their Supplemental Response mailed August 23, 2002. Applicants will first briefly describe the claimed invention, respond to the present Action with respect to the rebuttals to applicants' assertions, and further explain why Cabib et al. does not teach or suggest, either expressly or inherently, applicants' invention. In light of the discussion below, it is believed that the above rejections of record have been overcome and that all pending claims are in condition for allowance. Action toward this end is respectfully solicited.

B. The Invention

The present invention relates to methods for diagnosing dysplasia, pre-cancer or cancer *in situ* in biological tissue or cells of a living organism that include utilization of spectroscopic methods to analyze the metachromatic properties of various dyes in abnormal (e.g., dysplastic, pre-cancerous and cancerous) and normal cells. The inventors of the present invention have surprisingly discovered that the extent of the metachromatic shift observed in a dye from stained tissue or cells can be used to differentiate, for example, the aforementioned abnormal cells and/or tissues from normal cells and/or tissues. According to one embodiment of the methods described in the application, the metachromatic shift of a dye observed in the reflected light spectrum from a dye-stained test sample is compared to the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells wherein the diagnosis of the disease state of the reference cells or tissue upon which the previously obtained spectra are based was confirmed by conventional histopathology methods, such as histochemical methods. Thus, quicker, more precise diagnoses may be made according to the present method compared to existing methods.

C. Applicant's position

1. Applicant's assertions discussed in Supplementary Response (mailed August 23, 2002) to the Office Action mailed November 26, 2001

In applicants' above-mentioned Supplementary Response, applicants asserted that Cabib et al. do not expressly or inherently teach or suggest quantifying the extent of metachromatic shift of a metachromatic dye in a stained test sample and making a correlation with a dysplastic, pre-cancer or cancer disease state using this information as recited in the pending claims. The following three points were further discussed in great detail in the Supplementary Response, and support this assertion: (1) when the Cabib et al. reference is viewed as a whole, it is seen to primarily relate to detecting spatial organization and quantifying cellular and tissue natural constituents and does not teach or suggest methods of diagnosing dysplasia, pre-cancer or cancer by quantifying, for example, the metachromatic shift of a metachromatic dye; (2) Cabib et al. teach use of dyes as contrast agents to visualize structures (e.g., to look at biological components and/or their spectrum) and do not teach or suggest, either expressly or inherently, use of metachromasia for any diagnostic purpose; (3) one skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teaching of Cabib et al.

2. Response to current Action's assertions

a. Cabib et al. relate to detecting spatial organization and quantifying cellular and tissue natural constituents, and any method of diagnosing cancer discussed in Cabib et al. involves use of such information, not information relating to quantitating the metachromatic shift of a metachromatic dye

The Action appears to misunderstand applicants' assertion (1) above. The Action attempts to assert that the teachings of Cabib et al. include methods of diagnosing cancer, and asserts that applicants argue that Cabib et al. relate to detecting spatial organization or distribution and quantifying the cellular or tissue constituents, not

diagnosing cancer. However, on page 4 of applicants' Supplementary Response, lines 7-10, applicants stated "Cabib et al. relate to detecting spatial organization and quantifying cellular and tissue natural constituents and any method of diagnosing cancer discussed in Cabib et al. involves use of such information, not information relating to quantifying the metachromatic shift of a metachromatic dye." The emphasis on the methods of Cabib et al. being used to detect and quantify cellular and tissue natural constituents was to show that Cabib et al. are relying on the inherent spectra of biological components in making a determination of cancer, not use of dyes as described in applicants specification.

For example, applicants noted that column 1, lines 17-21, of Cabib et al. states that "[t]he methods of the present invention can be used to detect spatial organization (i.e., distribution) and to quantify cellular and tissue natural constituents, structures, organelles and administered components such as tagging probes (e.g., fluorescent probes) and drugs...." It is further seen in column 6, lines 36-39, of Cabib et al. that "[t]he method further enables the identification and spatial mapping of proteins, sacharides [sic], AND+ [sic] and NADH, collagen, elastin and flavin, and various additional metabolic mediators within cells and/or tissues." Additionally, it is stated in column 6, lines 27-33, that "[a]nother objective of the present invention is to map in a quantitative way white light, ultraviolet or laser-induced emission spectra from biological components (e.g., oxygenated and deoxygenated hemoglobin in retinal blood vessels and or melanin pigmentation level in the retina) and, to distinguish cancer from healthy, or otherwise diseased tissue or cells." **It will be appreciated from this statement that Cabib et al. are relying on the spectra of the biological components in making a determination of cancer.** This conclusion is buttressed by the methods discussed in Cabib et al. to map cancerous tissue as described in Example 7.

In the only example of mapping cancerous tissue *in vivo* (Example 7), which is a prophetic example, in colon, bladder, lungs, cervix and other internal organs, it is mentioned that the procedure is similar to the ophthalmologic examples in Example 6. It is mentioned, in column 58, lines 40-44, that the differences between the method of

Example 7 and Example 6 is in the collecting optics utilized, in the types of some basic molecular components involved in the detection: "some of these are probably common, such as oxygen concentration, additional others are collagen and elastin, genetic material in the cell nuclei, such as DNA chromatin, etc." Example 6 involves diagnosis of retinal abnormalities by measuring oxygen concentrations. However, it is also mentioned in column 56, lines 58-61, of Example 6 that, besides measuring oxygen by measuring the concentration of hemoglobin, "important information can be obtained also by measuring the concentration of other constituents, such as NAD^+, NADH, flavin, cytochromes, etc." No dyes are taught or suggested to be used in this example. Cabib et al. either rely on the inherent spectra of biological components, as discussed above, or the use of dyes as contrast agents as discussed on pages 11 and 12 of this response. As a matter of fact, the statements made in the Action to show that Cabib et al. teach methods of diagnosing cancer support applicants' assertion that the methods of Cabib et al. rely on the inherent spectra of biological components and the use of dyes as contrast agents in diagnosing cancer.

b. Passages from Cabib et al. cited in the Office Action, as well as the teachings of Cabib et al. as a whole, support the conclusion that the methods of Cabib et al. rely on the inherent spectra of biological components and the use of dyes as contrast agents in diagnosing cancer

It is asserted in the Action that the prior art (a) teaches "morphometric spectral image analysis enables evaluation of subtle cytological and histological features to yield useful ultrastructural and medical information for diagnostic and prognostic evaluation," (b) discloses "[s]ince various malignancies are also characterized by unique developmental features, the SpectraCube™ system and the methods of the present invention can be adopted to monitor these characterizing features and thus to assist in for example early diagnosis (e.g., existence and stage) of such malignancies;" and (c) discloses an example (Example 8) in which the SpectraCube™ system and the

methods of the claimed invention were used to differentiate a cancerous cell from a normal cell.

Turning now to assertion (a) above, the quote above relating to morphometric spectral image analysis was taken from the following paragraph:

> Spectral imaging applied to transmission light microscopy can greatly improve the quantitative measurement of size, shape and textural features of cells and tissues. This technique is known as morphometry, which is a rapidly growing field in biology and pathology [Erler et al. (1993) Modern Pathology, 6, p. 612-618]. *Morphometric spectral image analysis enables evaluation of subtle cytological and histological features to yield useful ultrastructural and medical information for diagnostic and prognostic evaluation* [Hytiroglou et al. (1992) Cancer 69, pp. 88-2121]. The ability to measure, quantitatively, the ratio of heterochromatic to euluomatin, [sic] as well as identifying different cell organelles by using spectral bio-imaging according to the methods of the present invention is demonstrated hereinbelow and in Example 2. (Sentence quoted in Office Action in italics).

The above-passage relates to applying spectral imaging to improve the quantitative measurement of the size, shape and textural features of cells. The analysis can be used to evaluate subtle cytological and histological features for diagnostic and prognostic evaluation. Example 2 is cited as an example of morphometric spectral image analysis. Example 2 involves measuring the ratio of heterochromatin to euchromatin (i.e., cellular structures) in stained tissue to study chromatin condensation. The dye is used as a contrast agent to visualize cellular structures.

In a further example of the use of dyes as contrast agents in Cabib et al., Cabib et al. mention in column 38, line 66 to column 39, line 9, that "[t]he spectral cytoplasmic features (spectrum B of FIG. 9a), when used for similarity mapping, allow the clear demarcation of components which one believe [sic] represent the nuclear envelope, Golgi cisternae, cytoplasmic vacuoles, and the outer cell membrane." Moreover, column 43, line 10, of Example 2 discloses that "[s]tandard analysis of blood cells is based on staining with either May-Grunwald-Giemsa or Romanowsky techniques which employ the dyes azure-B and Eosin." Such blood cell analysis involves examination of various cellular structures. For example, analysis of bone marrow cells, precursors to red blood cells, in Example 2 includes quantitation of euchromatin and heterochromatin and morphological analysis of cytoplasmic components and does not involve metachromasia.

Turning now to assertion (b) above relating to use of the SpectraCube™ system, the quoted passage is found at the end of Example 2, which, as discussed above, included quantitation of euchromatin and heterochromatin and morphological analysis of cytoplasmic components, and does not teach or suggest utilizing the metachromatic properties of a dye to diagnose cancer as recited in the pending claims.

Turning now to assertion (c), which states that the methods of Cabib et al. were used to differentiate a cancerous cell from a normal cell in Example 8, this example further buttresses applicants' assertion that Cabib et al. only teach use of dyes as contrast agents to visualize cellular structures. Example 8 of Cabib et al. teaches staining a cervical smear with haematoxylin-eosin for aiding in the diagnostic pathology as analyzed by a transmission microscopy RGB image. Haematoxylin-eosin is one of the most commonly used stains in histopathology. Haematoxylin is a basic dye that stains acidic structures (e.g., DNA, ribosomes and rough endoplasmic reticulum) a purplish-blue. Eosin is an acidic dye that stains basic structures red or pink (e.g., it stains basic cytoplasmic proteins pink or pinkish-red). **The stain is clearly used in this example as a contrast agent in order to quantitate various cellular structures to**

aid in the diagnosis. This staining procedure is commonly performed in the art and is not relevant to applicants' claimed invention.

For example, applicants have claimed a method for diagnosing dysplasia, pre-cancer or cancer *in situ* in biological tissues or cells of a living organism. *In situ* refers both to *in vivo* and *ex vivo* (i.e., freshly excised and otherwise living tissue). Prior to microscopically examining a Pap smear in the prior art, the sample is mounted on a slide, fixed and then stained. Such fixing significantly alters the underlying biochemistry of the cells/tissue such that the interaction of the stains is entirely different than if the stains are applied *in situ* to either freshly excised tissue or to a surgical site directly on the patient. The transverse thin sections are orthogonal to the cell membranes, leaving little of the membrane presented for viewing other than the edge. Conversely, the unique correlation with the metachromasia of the thiazine dyes with the disease state of the tissue as discovered by the inventors of the present invention relies on use of intact cells having an intact cell membrane. The unique sensitivity specifically of methylene blue and toluidine blue O, and thiazine dyes generally, for abnormal cells, such as pre-cancerous and cancerous cells, is due to the unique properties of the dyes (e.g., lipophilic, cationic, low molecular weight) which are readily taken up by the significantly modified cell membrane of such cells. For example, the enhanced membrane permeability of pre-cancerous and cancerous cells for these compounds appears to be in excess of the chemical activity needed for transport across a gradient potential. A fixed thin section neither has an active cell membrane potential to exclude or include the dye in an active manner, nor sufficient cell membrane presented to the viewer to determine the extent of dye localization.

Example 8 was discussed to show that the stain is clearly used in this example as a contrast agent in order to quantitate various cellular structures to aid in the diagnosis and that this staining procedure is commonly performed in the art and is not relevant to applicants' claimed invention. Applicants did not suggest or intend to suggest that Cabib et al. do not teach use of *in situ methods* as asserted in the Action, although it does not appear Cabib et al. teach use of freshly-excised tissue.

Additionally, in response to applicants' assertion that the prior art teaches that transmission microscopy suffers greatly from the inherently low contrast of cell organelles and structural details, the Action quotes column 36, lines 22-27, of Cabib et al. when stating "[t]he use of the spectral bio-imaging methods of the present invention is one of the most straightforward methods to increase the apparent contrast of cells and tissues examined under a transmission microscope." Applicants made the above-referenced statement to show that any mention of use of dyes in Cabib et al. relates to their use as contrast agents. Applicants stated that, in column 36, lines 17-22, of Cabib et al. it is mentioned that "[t]ransmission microscopy suffers greatly from the inherently low contrast of cell organelles and structural details. *Many methods have been developed to improve this contrast, among them staining* and spatial filtering." (Emphasis added). It will be appreciated, then, that the above-referenced passage was mentioned to support that applicants' position **Cabib et al. teach that staining is a method used to improve contrast**. Cabib et al. go on to discuss staining techniques to facilitate histological examination using organic stains which specifically bind to different macromolecules in cells and mention that the most common staining techniques are Romanowsky-Giemsa stain (eosin Y and azure B), and Haematoxylin-Eosin. Cabib et al. further mention, in column 37, lines 9-12, that "[w]hatever the technique, *with staining it is possible to distinguish between subcellular compartments of the cell and especially to distinguish the chromatin organization in the nucleus.*" (Emphasis added). These sections of Cabib et al., for example, show that dyes, if used at all, are used as contrast agents to, for example, quantitate various cellular structures.

c. Cabib et al. teach it is preferable not to use dyes in the methods described therein

Although Cabib et al. discuss use of stains in the methods described therein as contrast agents, it is discussed therein that is it preferable not to use dyes at all, thus supporting the conclusion that Cabib et al. do not teach or suggest that the dyes discussed in Cabib et al. have diagnostic value as found by the inventors of the present

invention. For example, it is mentioned in column 39, lines 13-16, of Cabib et al. that "[o]ne of the advantages of combining spectral bio-imaging and transmission microscopy is the ability to use a 'clean' measurement technique, i.e., no need for working with potentially toxic dyes or fixation agents." By contrast, the present inventors have recognized the diagnostic value of the dyes when employed according to the invention, and have taught the art how to extract, interpret and utilize this heretofore unappreciated information to advance human health.

In response to applicants' assertion that Cabib et al. teach that it is preferable not to use dyes at all in the methods described therein, the Action states that the Cabib et al. teach "*in some cases* there is no need to use potentially toxic dyes or fixation agents (column 39, lines 10-16)" (emphasis in original). With respect, this is not what is stated in the quoted passage. Column 39, lines 10-16, states:

> In some cases, spectral images acquired using transmission methods and unstained tissue may provide useful information, similar to that found in fluorescence microscopy techniques. One of the advantages of combining spectral bio-imaging and transmission microscopy is the ability to use a 'clean' measurement technique, i.e., no need for working with potentially toxic dyes or fixation agents.

Cabib et al. appear to be stating that prior art spectral images acquired using transmission methods and unstained tissue can provide useful information. However, Cabib et al. mention that the advantage of combining the spectral bio-imaging they describe and transmission microscopy is that there is no need for working with potentially toxic dyes or fixation agents. Therefore, they do not show that "in some cases, there is no need to use potentially toxic dyes or fixation agents" as asserted in the Action. After showing that Cabib et al., when viewed as a whole, teach use of stains in the methods described therein as contrast agents, applicants demonstrated that Cabib et al. teach it is preferable not to use dyes at all, supporting the conclusion that

Cabib et al. do not teach or suggest that the dyes discussed therein have diagnostic value as surprisingly found by the inventors of the present invention.

d. One skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teachings of Cabib et al.

Finally, the Action states that (a) "the artisan would understand that the dyes differentially stain cancerous and normal tissue such that the differences can be characterized by comparing the absorption or transmission spectra of the two types of dye-stained tissues"; (b) "the change in the absorption or transmission spectra of a dye, or the metachromatic shift is an inherent property of the dye"; and (c) "the prior art does teach methods that differentiate cancerous tissue and normal tissue on the basis of differences or similarities that are observed in the transmission spectra of a dye after staining a sample containing suspected cancerous cells and a library of tissues or cells that have been previously characterized as either cancerous or not. In practicing the method of the prior art, the artisan necessarily determined the metachromatic shift of the dye that was used to stain the tissues or cells."

Applicants have already stated their position that (1) when the Cabib et al. reference is viewed **as a whole**, it is seen to primarily relate to detecting spatial organization and quantifying cellular and tissue natural constituents, and any method of diagnosing cancer discussed in Cabib et al. involves use of such information, not information relating to quantifying the metachromatic shift of a metachromatic dye; (2) Cabib et al. teach use of dyes as contrast agents to visualize structures (e.g., to look at biological components and/or their spectrum) and do not teach or suggest, either expressly or inherently, use of metachromasia for any diagnostic purpose; and (3) one skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teaching of Cabib et al. Contrary to the assertions in the Action, the issue is not whether the change in absorption or transmission spectra of a dye, or the metachromatic shift, is an inherent property of the

dye, but whether the teachings of Cabib et al. as a whole teach or suggest the methods of the present invention.

Additionally, in order to establish inherency, the extrinsic evidence "'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill." *In re Roberston*, 49 U.S.P.Q.2d 1949, 1950-1951 (Fed. Cir. 1999) (citations omitted). "Inherency, however, may not be established by probabilities or possibilities. The mere fact that a given thing may result from a given set of circumstances is not sufficient.'" *Id.* (citations omitted). "In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic *necessarily* flows from the teachings of the applied prior art." *Ex parte Levy*, 17 U.S.P.Q.2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original). With respect, it is submitted that the Examiner has not met this burden for, *inter alia*, the following reasons.

There is no mention of toluidine blue O in the Cabib et al. Cabib et al. teach use of a stain composition that includes two dyes – eosin Y and either methylene blue (as in May-Grunwald-Giemsa) or azure B (as in Romanowsky-Giemsa). There is no teaching in Cabib et al. from which the person of ordinary skill could conclude that the method of Cabib et al., employing a combination of eosin Y and either methylene blue or azure B, inherently, that is, necessarily, utilizes a metachromatic shift to diagnose dysplasia, pre-cancer or cancer, because, *inter alia*, the dye compositions of Cabib et al. employ a non-metachromatic dye, and Cabib et al. teach a method based on contrast, not metachromasia. Moreover, one of ordinary skill in the art would not conclude from Cabib et al. that a metachromatic shift is necessarily and inherently taking place. In fact, it is only by the improper use of applicants' teaching that the Examiner can supply, in hindsight, the deficiencies of Cabib et al. Withdrawal of the rejection of claims 1, 5, and 7-11 under 35 U.S.C. § 102(e) is respectfully requested.

V. Rejection under 35 U.S.C. § 103(a)

Claims 1, 5, and 7-11 stand rejected as being unpatentable over U.S. Patent No. 5,784,162 to Cabib et al. in view of Tuite et al. (*Journal of Photochemistry and Photobiology B: Biology* 21:103-124, 1993), as evidence by Vaezy, et al. (*Journal of Microscopy* 163:85-94, 1991) and Marchesini, et al. (*Photochemistry and Photobiology* 55:515-522, 1992). Cabib et al. is relied on for teaching methods for using a combination of dyes comprising methylene blue, but, as the Examiner mentions, does not teach use of toluidine blue O. Tuite et al. is relied on for teaching that toluidine blue O can selectively stain tumor cells and is generally non-toxic to normal cells. The Action concludes that it would be obvious to have used toluidine blue O in the methods of Cabib et al. because both methylene blue and toluidine blue O had been characterized as non-toxic and are known to selectively stain cancer cells. It is further asserted in the Action that one of ordinary skill in the art would have been motivated to use toluidine blue in the methods of Cabib et al. to confirm the results of analyses in which methylene blue had been used.

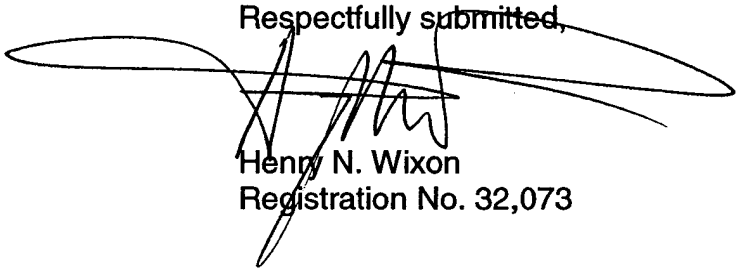
Applicants have already stated the deficiencies in the Cabib et al. reference in the previous section of this response, and Tuite et al. does nothing to correct the deficiencies of Cabib et al. Tuite et al. discuss photochemical interactions of methylene blue and analogues with DNA and other biological substrates. Specifically, methylene blue, azure B, Azure A, Azure C, thionine and toluidine blue O are discussed. As all these dyes are non-toxic and can selectively stain cancer cells, why would one skilled in the art select toluidine blue O to confirm the results of their analysis? It is respectfully submitted that any such selection is improperly based on hindsight, using the applicant's specification as a guide. Additionally, even if there were some motivation to select toluidine blue O to use in the methods of Cabib et al., such use would be as a contrast agent. There is no teaching or suggestion in any of the combined references of the method recited in the pending application. Withdrawal of the rejection of claims 1, 5, and 7-11 under 35 U.S.C. § 103(a) is respectfully requested.

VI. Conclusion

Cabib et al. do not teach or suggest, either expressly or inherently, the method as recited in the claims of the pending application. Withdrawal of the rejection of claims 1, 5 and 7-11 under 35 U.S.C. §§ 102(e), 103(a) and 112, first and second paragraphs, is respectfully requested.

In light of the foregoing discussion, it is believed that claims 1, 5 and 7-12 are in condition for immediate allowance. Action towards this end is respectfully requested. The Examiner is invited to telephone the undersigned attorney regarding any issues that may be handled in that fashion.

Respectfully submitted,



Henry N. Wixon
Registration No. 32,073

HALE AND DORR LLP
1455 Pennsylvania Avenue, N.W.
Washington, DC 20004-1008
(202) 942-8490
Date: May 19, 2003

APPENDIX 1

Please amend claims 1 and 10 as follows:

1. (Amended Four Times) A method for diagnosing dysplasia, pre-cancer or cancer *in situ* in biological tissue or cells of a living organism, comprising:

a) applying to the tissue or cells *in situ* a dye selected from the group consisting of methylene blue and toluidine blue O;

b) removing excess dye from the tissue or cells;

c) generating a reflected light spectrum from the tissue or cells by illuminating the stained tissue or cells with light;

d) directing the reflected light spectrum to a spectrometer;

e) comparing the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with the degree of the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells; and

f) correlating the reflected light spectrum with a disease state, <u>said disease state selected from the group consisting of dysplasia, pre-cancer and cancer,</u> whereby an *in situ* diagnosis of dysplasia, pre-cancer or cancer is made.

10. (Thrice Amended) A method as in claim 1, wherein the tissue[s] or cells are from at least one organ selected from the group consisting of skin, cervix, vagina, mouth, colon, <u>and</u> esophagus [and internal organs].

Please enter the following new claim:

12. (New) A method as in claim 1, wherein the tissue or cells are from an internal organ.

APPENDIX 2

1. (Amended Four Times) A method for diagnosing dysplasia, pre-cancer or cancer *in situ* in biological tissue or cells of a living organism, comprising:

a) applying to the tissue or cells *in situ* a dye selected from the group consisting of methylene blue and toluidine blue O;

b) removing excess dye from the tissue or cells;

c) generating a reflected light spectrum from the tissue or cells by illuminating the stained tissue or cells with light;

d) directing the reflected light spectrum to a spectrometer;

e) comparing the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with the degree of the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells; and

f) correlating the reflected light spectrum with a disease state, said disease state selected from the group consisting of dysplasia, pre-cancer and cancer, whereby an *in situ* diagnosis of dysplasia, pre-cancer or cancer is made.

5. A method as in claim 1, wherein said comparing comprises the use of a digital microprocessor.

7. A method as in claim 1, wherein the tissues or cells are thought to be metaplastic.

8. A method as in claim 1, wherein the spectrometer is able to measure light for a range of or some part of a range of wavelength from 200 to 1100 nanometers.

APPENDIX 2 (cont'd)

9. A method as in claim 1, wherein the reflected light spectrum is measured and recorded, and said measuring comprises the use of a photometer and one or more light filters.

10. (Thrice Amended) A method as in claim 1, wherein the tissue or cells are from at least one organ selected from the group consisting of skin, cervix, vagina, mouth, colon, and esophagus.

11. A method as in claim 1, wherein, prior to said comparing step, a reflected light spectrum from unstained tissue or cells is subtracted from the spectrum of the stained tissue or cells.

12. (New) A method as in claim 1, wherein the tissue or cells are from an internal organ.